CXII. THE KINETICS OF ALCOHOLIC FERMENTATION OF SUGARS BY BREWER'S YEAST.

II. THE RELATIVE RATES OF FERMENTATION OF GLUCOSE AND FRUCTOSE.

By REGINALD HAYDN HOPKINS
and RICHARD HENRY ROBERTS.

From the Department of Industrial Fermentation, University of Birmingham.

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The rate of fermentation of fructose by brewer's yeast was found by Slator [1908] to be the same as that of glucose over the range of conditions investigated. Hopkins [1931], however, observed that in low concentrations, less than 0·5 %, fructose was fermented appreciably more slowly than glucose at 30°. It was decided to extend the investigations on the effects of yeast rate and sugar concentration respectively (Exps. 2 and 3 reported in Part I of this series) to fructose, under conditions which permitted of comparison with glucose.

Experimental.

The apparatus and technique employed were exactly as described in Part I. Washed pressed yeast from the same source was used, and in Exp. 2 the glucose and fructose were fermented successively with yeast from the same sample.

Exp. 1. Variation of yeast rate.

Fructose 0·5 g. (1 %). Yeast 0·5–5·0 g.
Temp. 30°. Total volume 50 ml.

Table I. *Maximum rate of fermentation of fructose.*

Rates expressed as mg. CO_2 per minute.

Yeast rate (g.)	0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0
Maximum rate of fermentation	0·68	1·53	2·05	2·78	3·48	4·06	4·63	5·07	5·49	5·80

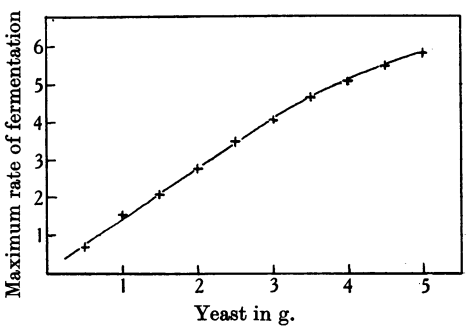

Fig. 1.

The graph, Fig. 1, shows proportionality between rate of fermentation and concentration of yeast only up to 3 g. in 50 ml., above which the fermentation rate is less than the proportional rate. This difference between the behaviour of fructose and that of glucose, which exhibited proportionality up to higher yeast rates, suggests that the yeast enzyme has a greater affinity for glucose than for fructose.

Exp. 2. Variation of initial concentration of glucose and fructose.

Glucose or fructose 0·1–15 %. Yeast 1 g.
Temp. 30°. Total volume 100 ml.

The rates of fermentation in solutions of various initial concentrations are set out in Table II. The results given correspond to certain arbitrary time stages and the constant rates calculated from the manometer readings over the period of approximate constancy are summarised in Table II *a*.

Table II. *Progressive rates of fermentation of glucose and fructose at varying initial concentrations.*

Rates expressed as mg. CO_2 per minute.

	Time (mins.)...	5	10	20	30	40	50
Glucose	0·1 %	—	0·74	0·62	0·59	—	—
	0·2 „	1·07	1·07	1·09	1·09	—	—
	0·3 „	1·13	1·22	1·33	1·36	—	—
	0·4 „	—	1·41	1·41	1·47	—	—
	0·5 „	—	1·44	1·55	1·58	—	—
	1·0 „	—	1·58	1·58	1·75	1·81	1·77
	2·0 „	—	1·41	1·58	1·93	1·98	1·98
	4·0 „	—	—	—	2·06	2·09	2·15
	6·0 „	—	—	1·81	2·04	2·15	2·17
	8·0 „	—	—	1·75	2·06	2·09	2·18
	10·0 „	—	—	1·92	2·04	2·04	2·05
	15·0 „	—	—	1·70	1·74	1·93	1·98
Fructose	0·1 „	—	0·48	0·45	0·42	—	—
	0·2 „	—	0·79	0·71	0·68	—	—
	0·3 „	—	0·85	0·91	0·91	—	—
	0·4 „	—	1·07	1·02	1·02	—	—
	0·5 „	—	1·19	1·24	1·16	—	—
	1·0 „	—	1·41	1·58	1·58	—	—
	2·0 „	1·58	1·64	1·78	1·78	—	—
	4·0 „	1·65	1·91	1·98	1·98	—	—
	6·0 „	1·60	1·77	1·87	2·01	1·98	—
	8·0 „	1·65	1·84	1·98	2·07	2·12	—
	10·0 „	1·56	1·56	1·77	1·77	—	—
	15·0 „	1·13	1·41	1·44	1·41	—	—

Table II*a*. *Constant rates of fermentation of glucose and fructose at varying initial concentrations.*

Rates expressed as mg. CO_2 per minute.

Concentration of sugar %	0·1	0·2	0·3	0·4	0·5	1·0	2·0	4·0	6·0	8·0	10·0	15·0
Rate, glucose	0·71	1·09	1·31	1·44	1·55	1·77	1·95	2·10	2·17	2·12	2·05	1·85
Rate, fructose	0·45	0·71	0·90	1·05	1·15	1·55	1·78	1·94	1·96	2·00	1·77	1·42

The results in Table II *a* are recorded graphically in Fig. 2 in which rates of fermentation are plotted as ordinates and concentrations as abscissae.

The results confirm those of Slator [1906] and Hopkins [1931]. Maximum rates of fermentation were obtained in solutions in the neighbourhood of 6 % initial concentration, and it is to be observed that whilst the maximum rates for the respective sugars differ but little, nevertheless the fructose curve lies below that of glucose throughout its length.

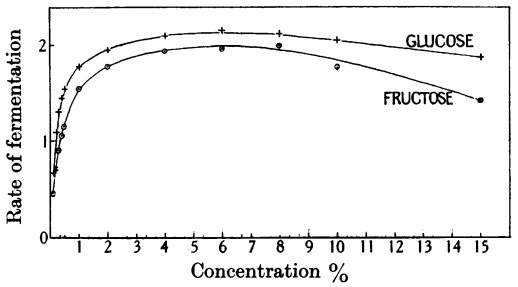

Fig. 2.

Discussion.

Application of the Michaelis and Menten [1913] equation,

$$\frac{1}{v}=\frac{K_m}{V}\cdot\frac{1}{x}+\frac{1}{V}$$

(see Part I), to the fructose results yielded the values recorded in Table III and plotted in Fig. 3 *a*.

Table III. *Fructose.*

x = conc. %	v = rate	$1/x$	$1/v$
0·2	0·71	5·00	1·408
0·4	1·06	2·50	0·943
0·6	1·27	1·67	0·787
0·8	1·43	1·25	0·699
1·0	1·54	1·00	0·649
1·2	1·60	0·83	0·625
1·4	1·66	0·71	0·602
1·6	1·70	0·63	0·588
1·8	1·74	0·56	0·575
2·0	1·78	0·50	0·562
2·2	1·80	0·45	0·556
2·4	1·82	0·42	0·550
2·6	1·85	0·38	0·541
2·8	1·87	0·36	0·535
3·0	1·89	0·33	0·529
3·5	1·92	0·29	0·521
4·0	1·95	0·25	0·513
6·0	2·00	0·17	0·500
10·0	1·84	0·10	0·544
15·0	1·42	0·07	0·704

As with glucose, the results of which were similarly dealt with in Part I (Table IV, Fig. 3 *a*), the graph is a straight line over a large part of its length. In the case of fructose this is true between the abscissa values corresponding to 0·4 % and 6 % concentration, indicating that the theory is valid over this range. In the case of glucose the corresponding limits were 0·2 % and 5·0 %.

In the cases of both glucose and fructose, it is possible to deduce the values of V, the maximum velocity of fermentation, and of K_m, the dissociation constant of the enzyme-substrate compound.

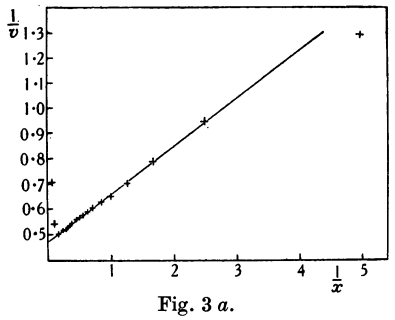

Fig. 3 *a*.

When x is large, *i.e.* when $\frac{1}{x}$ becomes zero, then

$$\frac{1}{v}=\frac{1}{V} \text{ and } v=V.$$

The value of $1/V$ can be read off from the graph where $1/x$ equals zero.

In the cases of both fructose and glucose the value of $1/V$ is the same and equals 0·47.

V is therefore 2·13.

Since K_m/V represents the slope of the curve, then

(*a*) Glucose

$$\text{Slope of line}=\frac{0\cdot92-0\cdot47}{5}=0\cdot090=\frac{K_m}{V}.$$

Hence $K_m=0\cdot090\times2\cdot13$

$=0\cdot192$, corresponding to $0\cdot01067\,M$.

(*b*) Fructose

$$\text{Slope of line}=\frac{1\cdot23-0\cdot47}{4}=0\cdot190=\frac{K_m}{V}.$$

Hence $K_m=0\cdot190\times2\cdot13$

$=0\cdot405$, corresponding to $0\cdot0225\,M$.

These values of K_m, 0·192 and 0·405 for glucose and fructose respectively are in fairly close agreement with those deduced by Dawson [1932] from the results of Hopkins [1931], 0·2 and 0·4, assuming the applicability of the Michaelis and Menten theory to fermentation by living yeast.

The values of K_m and V have significance in that from them can be calculated the so-called "selectivity constant," *i.e.* that which is derived from the results of selective fermentations of mixtures of the two sugars, Hopkins [1931]. Thus it was shown by Michaelis and Menten [1913] that the relative rate of reaction of two substrates competing for the same enzyme is given by the equation

$$\frac{V_a}{V_b}\cdot\frac{K_a}{K_b}=K \text{ (selectivity constant)},$$

where K_a, K_b=the Michaelis constants of the two substrates, V_a, V_b=the theoretical maximum velocities with each substrate alone.

Substituting the values of V and K obtained above for glucose and fructose we get

$$K=\frac{2\cdot13}{2\cdot13}\cdot\frac{0\cdot405}{0\cdot192}=2\cdot10=K_{G/F},$$

which is again in close agreement with the value (2) calculated by Dawson from the experimental results of Hopkins.

The value of V, the maximum rate of fermentation deduced above (2·13, the number of mg. of CO_2 evolved per minute under the conditions of Exp. 2) is the same for both glucose and fructose. This means that for both sugars the theoretical maximum rate of fermentation is the same. In practice this is not attained since apparently hypertonic and other effects supervene.

Application of the equation, $\log v = \log k + n \log$ [sugar] as applied to fermentation of glucose (see Part I, Table V), gives the results in Table IV, columns 1 and 2, which are plotted in Fig. 3 *b*. From the slope of the curve are calculated the values of n given in column 4, corresponding to the concentrations of fructose in column 3. These values are represented graphically in Fig. 3 *c*. In both Figs. 3 *b* and 3 *c* the corresponding curves for glucose are reproduced for comparison (from Figs. 3 *b* and 3 *c* of Part I).

Table IV.

Log (concentration)	Log (rate of fermentation)	Concentration %	n
-0·699	-0·149	—	—
-0·523	-0·046	0·30	0·52
-0·398	0·021	0·40	0·49
-0·301	0·061	0·50	0·45
0	0·190	1·0	0·30
0·301	0·250	2·0	0·16
0·602	0·288	4·0	0·045
0·778	0·301	6·0	-0·035
0·903	0·292	8·0	-0·16
1·000	0·265	10·0	-0·30
1·176	0·152	—	—

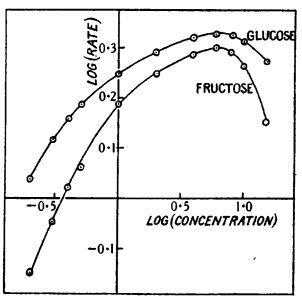

Fig. 3 *b*.

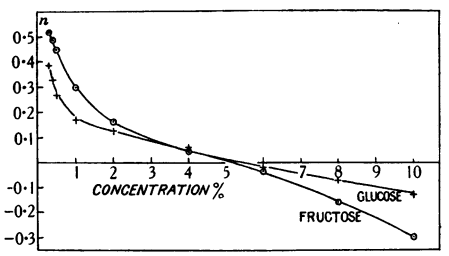

Fig. 3 *c*.

Between concentrations of 2 and 8 % fructose the value of n approximates to zero, and the rate of fermentation as given by the equation $v = k$ [fructose]^n is approximately independent of the concentration of sugar. The corresponding limits for glucose concentration are 1 and 10 %.

Certain interesting differences between the behaviours of the two sugars are illustrated by these curves. The dependence of rate of fermentation on concentration of sugar is, at low values of the latter more marked in the case of fructose than of glucose. This is in agreement with the higher deduced value of K_m for fructose, for which sugar the yeast enzyme has less affinity than for glucose. This is confirmed by the results of the experiments on the effect of yeast rate. At high concentrations of sugar the rate of fermentation of fructose never quite equals that of glucose, since, apparently, hypertonic and/or other effects supervene. It is, however, evident that there exists a greater difference

between the kinetics of fermentation of glucose and fructose than would be supposed from a superficial consideration of the results obtained by earlier workers.

SUMMARY.

1. Proportionality between the yeast rate and rate of fermentation of fructose exists up to a certain concentration of yeast, above which the fermentation rate is less than proportional. With glucose proportionality exists up to higher yeast rates.

2. Under selected identical conditions glucose is fermented at approximately the same rate between 1 and 10 %, and fructose between 2 and 8 % concentration, the rate for fructose being slightly less than that for glucose. Below these concentrations fructose is fermented much more slowly than glucose.

3. Under the conditions of experiment adopted, the theory of Michaelis and Menten is valid for the fermentation of glucose between 0·2 and 5 % and of fructose between 0·4 and 6 % concentration.

4. The values of the dissociation constants (K_m) of the compound of enzyme and substrate are 0·01067 and 0·0225 for glucose and fructose respectively, and the ratio of these gives the value for $K_{G/F}=2·10$ for the selectivity constant in agreement with the known value of this constant derived from experiments on selective fermentation of mixtures of the sugars by similar brewery yeasts.

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